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14. ABSTRACT

Identification of genes driving prostate carcinogenesis will lead to new cancer treatment. The human chromosome 8q24.21 region has been linked with increased risk for prostatic carcinoma but the how this region contributes to prostate carcinogenesis is unknown. We cloned a candidate gene, *POU5F1B* (also called POU5F1P1), in this gene desert of 1.2Mb between *FAM84B* and the *c-MYC* oncogene. *POU5F1B* is a pseudogene of embryonic Oct4 (POU5F1). A recent study found that tumor Oct4 found in prostate cancer cells is due to the gene expression of POU5F1B, not embryonic Oct4 (POU5F1). In a dataset of 171 patients, it was found that tumor Oct4 was significantly increased in primary tumors and markedly increased in metastatic tumors, when compared to normal prostate or adjacent normal tissues. Based on the analyses and our preliminary data, we think, tumor Oct4, expressed from POU5F1B in the prostate cancer susceptibility loci 8q24, is a driver of prostate tumor formation and progression, and therefore, this driver is a novel target of intervention to eliminate prostate cancer. We propose to further determine the roles of tumor Oct4 in prostate tumor formation and metastasis. We hope we can validate whether tumor Oct4 can be targeted to inhibit prostate cancer progression and metastasis. In addition, we will map out the regions critical for Oct4 to promote prostate carcinogenesis so that we can target this region to develop therapeutics for cancer treatment in the future

15. SUBJECT TERMS
OCT4, cancer stem cells, prostate cancer, metastasis, tumor formation

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Introduction

Background: Genome-wide association studies (GWAS) have linked human chromosome 8q24.21 region with increased risk for prostatic carcinoma but the how this region contributes to prostate carcinogenesis is unknown. In this gene desert of 1.2Mb between *FAM84B* and the *c-MYC* oncogene, *POU5F1B* (also called POU5F1P1) is a candidate gene with coding capacity. It is a pseudogene of embryonic Oct4 (POU5F1). A recent study found that tumor Oct4 found in prostate cancer cells is due to the gene expression of POU5F1P1 (Hugo name: POU5F1B), not embryonic Oct4 (POU5F1). Our *in silico* analysis found a significant increase in Oct4 (*POU5F1B*) in primary tumors and a marked increase in metastatic tumors, when compared to normal prostate or adjacent normal tissues. Tumor Oct4 expression was higher in tumorigenic prostate cancer cells than in non-tumorigenic RWPE-1 cells. Depletion of tumor Oct4 in prostate cancer cells reduced their tumorigenic potential. We cloned tumor Oct4 and found that increased expression of tumor Oct4 in prostate cancer cells stimulated tumor cell motility. Further a significant divergence was found between tumor Oct4 and embryonic Oct4 in regulating Wnt/β-caenin signaling. It is our hypothesis that tumor Oct4, expressed from POU5F1B in the prostate cancer susceptibility loci 8q24, is a driver of prostate tumor formation and progression, and therefore, this driver is a novel target of intervention to eliminate prostate cancer.

Objective: The objective is to determine whether tumor Oct4 promotes tumor formation and metastasis, to determine whether tumor Oct4 can be targeted to treat prostate cancer progression, and to elucidate the mechanism involved for tumor Oct4 to promote prostate carcinogenesis.

Specific Aims: 1) Investigate whether tumor Oct4 promotes prostate tumor initiation and metastasis.

2) Determine whether tumor Oct4 can be targeted to reduce prostate tumor formation, progression, and metastasis.

3) Elucidate the mechanism involved for tumor Oct4 in promoting prostate carcinogenesis.

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BODY OF REPORT

Scientific portion:

Task 1. Investigate whether tumor Oct4 promotes prostate tumor initiation and metastasis (Month 1 – 18).

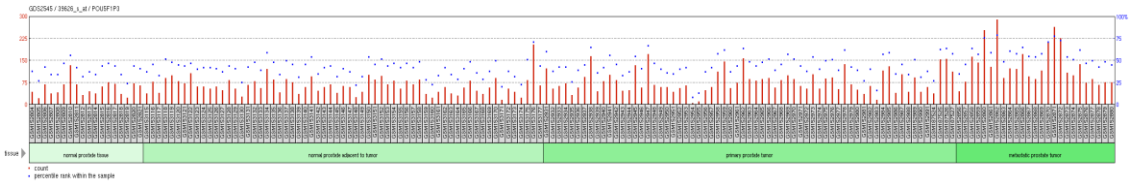
The overexpression of tumor POU5F1B in prostate cancer LNCaP cell lines and subsequent effects on tumor cell growth in vitro and tumor formation and growth in vivo have been described in last report. Here we describe some findings previously not reported.

1.1 POU5F1B expression in prostatic tissue

As cancer stem cell marker, OCT4 expression has been observed in many cancers, including breast cancer(Ezeh et al., 2005), bladder cancer(Atlasi et al., 2007), lung cancer(Karoubi et al., 2009) and prostate cancer(Monsef et al., 2009; Sotomayor et al., 2009; Su et al., 2004). However, a recent conclusive study suggests that POU5F1B, not OCT4-A or OCT4-B, is expressed in prostate cancer cell lines and prostatic tissue(Kastler et al., 2010).

To access the clinical relevance of POU5F1B in prostate carcinogenesis, we conducted *in silico* analysis of expression profiles in a prostate cancer progression dataset (GDS2545). The dataset were obtained from 171 samples including normal, adjacent normal, primary tumors, and metastatic tumors (From left to right, Figure 1A). A general trend of increased POU5F1B expression was noted. Further detailed analysis of the probe intensity in the four types of tissues revealed a significant increase in POU5F1B in primary tumors and a marked increase in metastatic tumors, when compare to normal prostate or adjacent normal tissues (Figure 1B). It should be noted that in this dataset no POU5F1 (OCT4) expression was found. The data suggest that POU5F1B, not POU5F1 (OCT4), is responsible for prostate cancer progression.

(A)



(B)

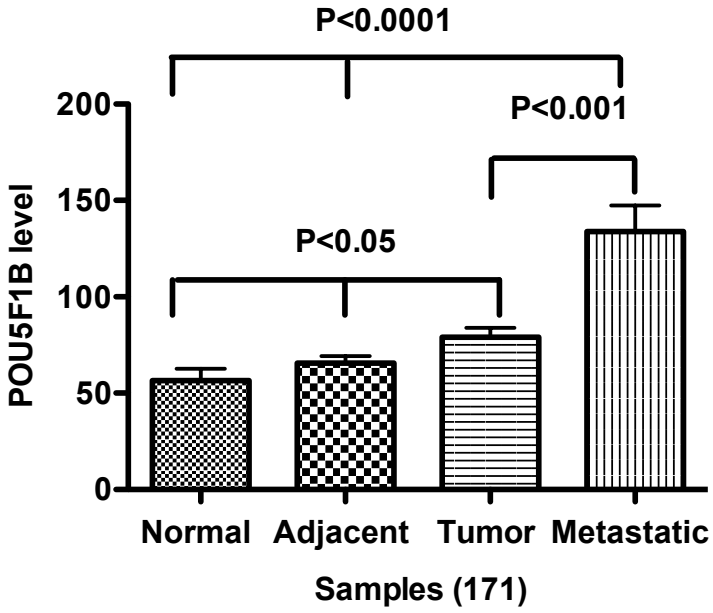


Figure 1. *In silico* analysis of POU5F1B expression in prostatic tissue.

(A) GEO profile of POU5F1B in Metastatic prostate cancer (HG-U95A). Dataset type: expression profiling by array, count, 171 samples.

(B) POU5F1B expression profiles during prostate cancer progression (GDS2545/39626_s_at/POU5F1B/Homo sapiens). Note the significant increase in POU5F1B expression in prostate tumors, especially in metastatic tumors, when compared to adjacent normal tissue or normal prostate tissue.

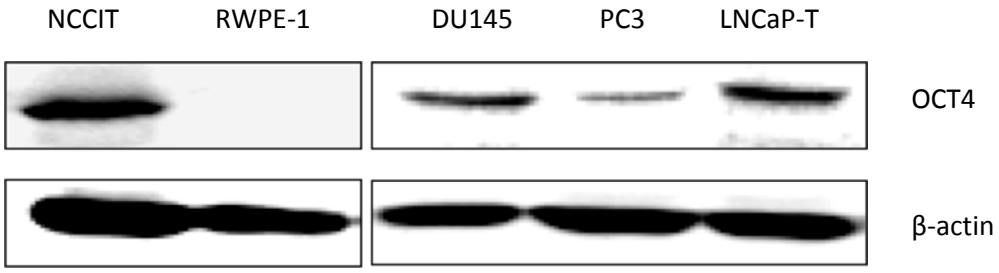
1.2 POU5F1B expression in prostate cell line and prostate carcinoma tissue

So far, six pseudogenes for OCT4 have been identified. The similarity between them and OCT4 are very high, three of them have >97%(Suo et al., 2005). The highly homologous between OCT4 and its pseudogenes can cause false positive artifacts of OCT4 expression by RT-PCR(Liedtke et al., 2008). By using OCT4 and POU5F1B specific RT-PCR, Kalster *et al.* showed it is POU5F1B, but not OCT4 is expressed in prostatic carcinoma and surrounding prostatic tissue(Kastler et al., 2010).

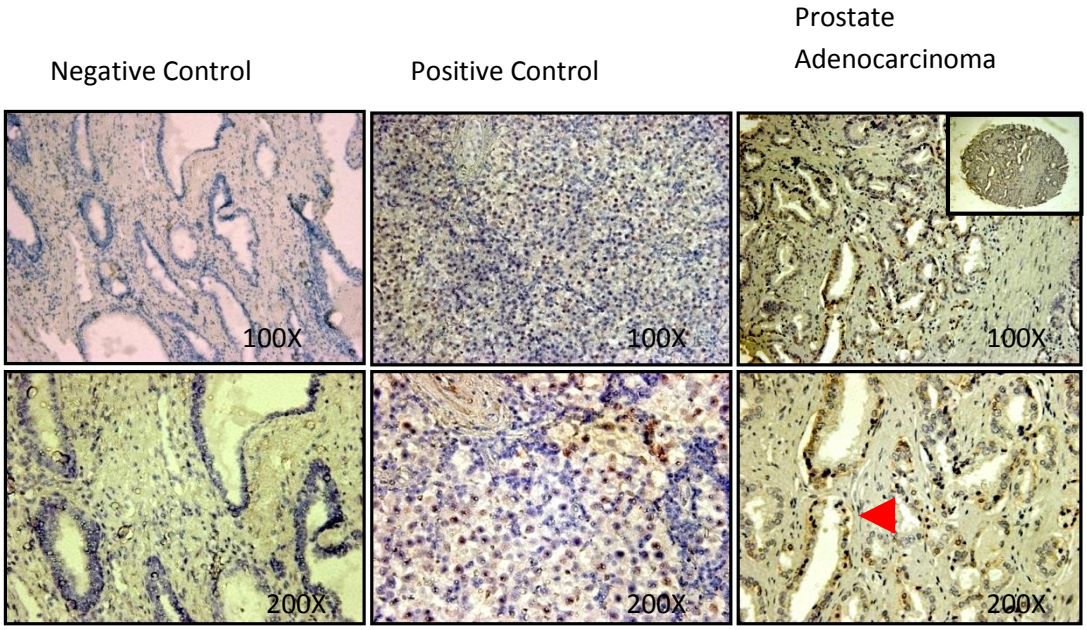
We can use OCT4 antibody to detect POU5F1B in prostate cell lines. First, we investigate the POU5F1B protein level in prostate tumor cell lines. Immunoblot showed POU5F1B was present in DU145, PC3, and LNCaP-T cells, a highly tumorigenic sublines of LNCaP cells. Embryonic teratoma cell, NCCIT, was used as positive control to determine the target protein band. Interestingly, POU5F1B was not detected in RWPE-1 cells, HPV-immortalized normal prostate epithelial cells (Figure 2 A).

Further, we examined POU5F1B expression in prostate tumor tissue by immunohistochemistry (IHC) using a validated monoclonal antibody Cell Marque MRQ-10). As shown Figure 2 B**,** nuclear localized Oct3/4 positivity was present in the positive control (Cell Marque; CXS121). Among 44 prostate tumor tissues examined, nine had positive staining for POU5F1B. There was only one tumor section positive for POU5F1B staining out of seven prostate cancer patient specimens with the Gleason score less than three (14%), and three from 21 malignant specimens with Gleason scores from five to seven (14%). The other five tissues positive for 4 POU5F1B were from 16 prostate adenocarcinomas with Gleason scores greater than eight (31%), suggesting an increased incidence of POU5F1B positivity in high-grade prostate adenocarcinomas. In addition, the positive staining for POU5F1B in tumor specimen was mainly localized in the cell nucleus.

(A)


NCCIT
RWPE-1
DU145
PC3
LNCaP-T
OCT4
β-actin

(B)


Negative Control
Positive Control
Prostate Adenocarcinoma
100X
100X
100X
200X
200X
200X

(C)

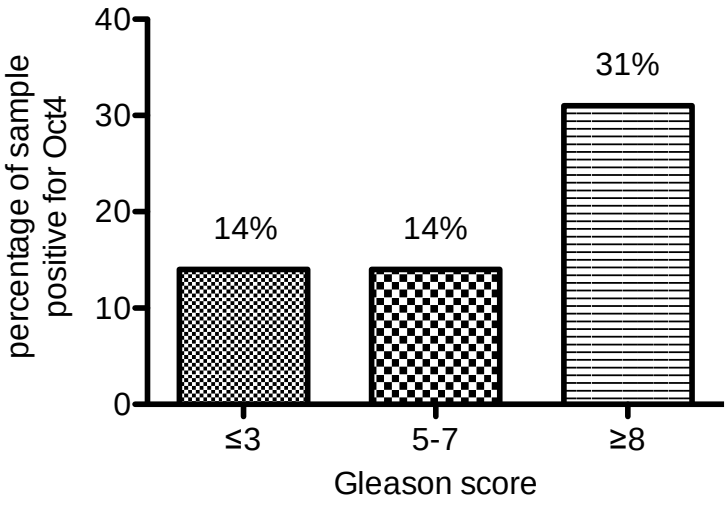

percentage of sample positive for Oct4
40
30
20
10
0
14%
14%
31%
≤3
5-7
≥8
Gleason score

Figure 2. POU5F1B expression in prostate cell lines and prostate carcinoma tissue.

(A) POU5F1B expression in prostate cell lines. Western blot analysis of POUF1B expression in human embryonic teratoma cell NCCIT, immortalized prostate epithelial cell RWPE-1, and prostatic carcinoma DU145, PC3, and LNCaP cells. NCCIT was used as the positive control for OCT4 expression.

(B) Immunohistochemistry analysis of POU5F1B in prostate carcinoma samples. Left panel is the negative control. Middle panel showed positive control (Cell MARQUE, CXS121) of POU5F1B. Right panel is a malignant prostate carcinoma tissue D16 (Gleason score= 1+2) with expression of POU5F1B. Brown color indicates positive staining. Note the nuclear localization of the staining.

(C) Statistical analysis of POU5F1B expression in 44 prostate tumor tissues. The increased incidence of POU5F1B positive staining in higher grade prostate adenocarcinomas was noted.

1.3 Alignment of PC3 POU5F1B ORF sequence with NCBI POU5F1B sequence (NM_001159542.1)

The enzyme digestion confirmed constructs were sent for sequencing. When we aligned the sequence of insert with POU5F1B (NM_001159542.1), two mismatches was noted, $CAG^{684} \rightarrow CAA^{684}$ (Glutamine/Q→Glutamine/Q) and $G^{712}AG \rightarrow C^{712}AG$ (Glutamic acid/E→Glutamine/Q). The first mismatch is a silent mutation, which will not cause amino acid change. The second mismatch will cause glutamic acid to glutamine change (Figure 3A). These two mismatches have been reported as SNP rs6998254 and SNP rs7002225 respectively (Figure 3B).

When compare PC3 POU5F1B amino acid sequence with NCBI POU5F1B amino acid sequence, only one amino acid changed, E^{238} in NCBI POU5F1B but Q^{238} in PC3 POU5F1B. When compare POU5F1B amino acid with POU5F1, we found fifteen amino acids are different, eight of them located at N domain, two located at POU specific domain, one in linker region, one in POU homeodomain and three in C domain

(Figure 3C). Some amino acids change may contribute to protein structure, such as R33L, from basic, positive, polar to neutral hydrophobic; G97S, hydrophobic to polar; D108N, acid, negative to neutral; T118P, polar to hydrophobic; E135K, acidic, negative to basic, positive; T170I, polar to hydrophobic; T182K, neutral to basic, positive; Q259 polar was deleted; T351I, polar to hydrophobic (Table 1). Hans R. Schöler showed the linker between two POU domains of mouse OCT4 is exposed to the surface of the protein and it is very important for reprogramming activity of OCT4 and protein-protein interaction(Esch et al., 2013). To map the sequences or residues which are critical for the different function between POU5F1B and OCT4 would provide more clues for better understanding why it is POU5F1B not OCT4 is expressed in prostatic carcinoma and surrounding prostatic tissue.

(A)

```
Insert   661   TGCAAAGCAGAAACCCTCATGCAAGCCCGAAAGAGAAAGCGAACCAGTATCCAGAACCGA  720
               ||||||||||||||||||||||| |||||||||||||||||||||||||| ||||||||
POU5F1B  661   TGCAAAGCAGAAACCCTCATGCAGGCCCGAAAGAGAAAGCGAACCAGTATCGAGAACCGA  720

Insert   721   GTGAGAGGCAACCTGGAGAATTTGTTCCTGCAGTGCCCGAAACCCACACTGCAGATCAGC  780
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
POU5F1B  721   GTGAGAGGCAACCTGGAGAATTTGTTCCTGCAGTGCCCGAAACCCACACTGCAGATCAGC  780

Insert   781   CACATCGCCCAGCAGCTTGGGCTCGAGAAGGATGTGGTCCGAGTGTGGTTCTGTAACCGG  840
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
POU5F1B  781   CACATCGCCCAGCAGCTTGGGCTCGAGAAGGATGTGGTCCGAGTGTGGTTCTGTAACCGG  840
```

(B)

rs6998254 *[Homo sapiens]*

21.

TATGCAAAGCAGAAACCCTCATGCA[A/G]GCCCGAAAGAGAAAGCGAACCAGTA

Chromosome:	8:127416550
Gene:	LOC101930033 (GeneView) POU5F1B (GeneView)
Functional Consequence:	intron variant,synonymous codon
Validated:	by 1000G,by 2hit 2allele,by cluster,by frequency
Global MAF:	G=0.4793/1044

rs7002225 *[Homo sapiens]*

22.

CCGAAAGAGAAAGCGAACCAGTATC[C/G]AGAACCGAGTGAGAGGCAACCTGGA

Chromosome:	8:127416578
Gene:	LOC101930033 (GeneView) POU5F1B (GeneView)
Functional Consequence:	intron variant,missense
Validated:	by 1000G,by 2hit 2allele,by cluster,by frequency,by hapmap
Global MAF:	C=0.4959/1080

(C)

```
POU5F1          1   MAGHLASDFAFSPPPGGGGDGPGGPEPGWVDPRTWLSFQGPPGGPGIGPGVGPGSEVWGI  60
POU5F1B         1   MAGHLASDFAFSPPPGGGGDGPWGAEPGWVDPLTWLSFQGPPGGPGIGPGVGPGSEVWGI  60
PC3 POU5F1B     1   MAGHLASDFAFSPPPGGGGDGPWGAEPGWVDPLTWLSFQGPPGGPGIGPGVGPGSEVWGI  60

POU5F1         61   PPCPPPYEFCGGMAYCGPQVGVGLVPQGGLETSQPEGEAGVGVESNSDGASPEPCTVTPG  120
POU5F1B        61   PPCPPPYELCGGMAYCGPQVGVGLVPQGGLETSQPESEAGVGVESNSNGASPEPCTVPPG  120
PC3 POU5F1B    61   PPCPPPYELCGGMAYCGPQVGVGLVPQGGLETSQPESEAGVGVESNSNGASPEPCTVPPG  120

POU5F1        121   AVKLEKEKLEQNPEESQDIKALQKELEQFAKLLKQKRITLGYTQADVGLTLGVLFGKVFS  180
POU5F1B       121   AVKLEKEKLEQNPEKSQDIKALQKELEQFAKLLKQKRITLGYTQADVGLILGVLFGKVFS  180
PC3 POU5F1B   121   AVKLEKEKLEQNPEKSQDIKALQKELEQFAKLLKQKRITLGYTQADVGLILGVLFGKVFS  180

POU5F1        181   QTTICRFEALQLSFKNMCKLRPLLQKWVEEADNNENLQEICKAETLVQARKRKRTSIENR  240
POU5F1B       181   QKTICRFEALQLSFKNMCKLRPLLQKWVEEADNNENLQEICKAETLMQARKRKRTSIENR  240
PC3 POU5F1B   181   QKTICRFEALQLSFKNMCKLRPLLQKWVEEADNNENLQEICKAETLMQARKRKRTSIQNR  240

POU5F1        241   VRGNLENLFLQCPKPTLQQISHIAQQLGLEKDVVRVWFCNRRQKGKRSSSDYAQREDFEA  300
POU5F1B       241   VRGNLENLFLQCPKPTL*QISHIAQQLGLEKDVVRVWFCNRRQKGKRSSSDYAQREDFEA  299
PC3 POU5F1B   241   VRGNLENLFLQCPKPTL*QISHIAQQLGLEKDVVRVWFCNRRQKGKRSSSDYAQREDFEA  299

POU5F1        301   AGSPFSGGPVSFPLAPGPHFGTPGYGSPHFTALYSSVPFPEGEAFPPVSVTTLGSPMHSN  360
POU5F1B       300   AGSPFSGGPVSFPPAPGPHFGTPGYGSPHFTALYSSVPFPEGEVFPPVSVITLGSPMHSN  359
PC3 POU5F1B   300   AGSPFSGGPVSFPPAPGPHFGTPGYGSPHFTALYSSVPFPEGEVFPPVSVITLGSPMHSN  359
```

Figure 3. Alignment of PC3 POU5F1B with NCBI POU5F1B (NM_001159542.1) and NCBI POU5F1 (NM_002701.5).

(A) Alignment of insert sequence cloned from PC3 cDNA with NCBI POU5F1B (NM_001159542.1) sequence. 2 mismatches were found as showed in red boxes.

(B) Two reported SNPs in POU5F1B gene. SNP rs6998254 showed CAG^{684}→CAA^{684} variant allele, and SNP rs7002225 showed $G^{712}AG$→$C^{712}AG$ variant allele.

(C) Alignment of PC3 POU5F1B putative amino acid sequence with NCBI POU5F1B putative amino acid sequence and NCBI POU5F1 amino acid sequence. Yellow highlight indicate POU specific domain, green highlight indicate POU homeodomain, and blue highlight indicate linker between these two domains. Amino acids in red mean differences between them.

Table 1 Different amino acid between POU5F

Location	OCT4	POU5F1B/PC3 POU5F1B	Domain
22	G	W	N domain
24	P	A	N domain
33	R (Basic, Positive, Polar)	L (Neutral, Hydrophobic)	N domain
69	F	L	N domain
97	G (Hydrophobic)	S (Polar)	N domain
108	D (Acidic, Negative)	N (Neutral)	N domain
118	T (Polar)	P (Hydrophobic)	N domain
135	E (Acidic, Negative)	K (Basic,, Positive)	N domain
170	T (Polar)	I (Hydrophobic)	POUs domain
182	T (Neutral)	K (Basic, Positive)	POUs domain
227	V	M	Linker
238	E(negative, acidic)	E/Q (Neutral)	POUh domain
259	Q (Polar)	-	POUh domain
314	L	P	C domain
344	A	V	C domain
351	T (Polar)	I (Hydrophobic)	C domain

1.4. POU5F1B can promote colony formation in soft agar

The anchorage-independent growth (AIG) is an important step in acquisition of malignancy(Freedman and Shin, 1974). Cells with anchorage-independent growth ability have the potential to migrate through the body, colonize other tissues and grow metastatically(Gassmann and Haier, 2008). The soft agar colony formation assay is a common method to monitor anchorage-independent growth.

As showed in Figure 4, LNCaP POU5F1B formed 75.5±7.63 colonies in soft agar, whereas in LNCaP pCDH-myc vector, the number of colonies was significantly decreased, only 26.75±7.63 (P<0.001).

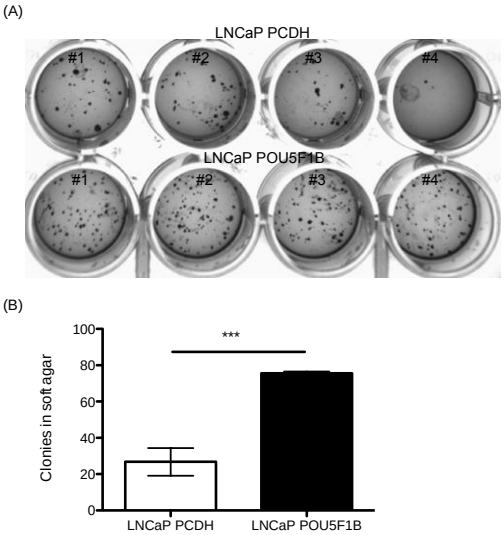


Figure 4. POU5F1B can promote colony formation in soft agar in LNCaP cells.

(A) LNCaP pCDH vector and LNCaP POU5F1B cells were seeded on soft agar and cultured for 7 days. The stained colonies were photographed, and counted.

(B) Statistical data of clonies formed in soft agar. 4 repeats for each group. P<0.001.

1.5 POU5F1B changes cell morphology in DU145 cells

We noticed that DU145 cells with POU5F1B overexpression have different cell morphology when compared with DU145-pCDH myc vector cells. DU145 pCDH-myc vector showed tightly packed and formed clustered structures, typical of epithelial cells and suggestive of strong cell-cell adhesion (Figure 5 left panel). DU145 cells overexpressed POU5F1B showed reduced cell-cell adherens junctions and suggestive of increased cell molitity (Figure 5 right panel). This observation are more significant under 200 magnification. This morphology change is consistant with previous data which showed POU5F1B can promote cell migration.

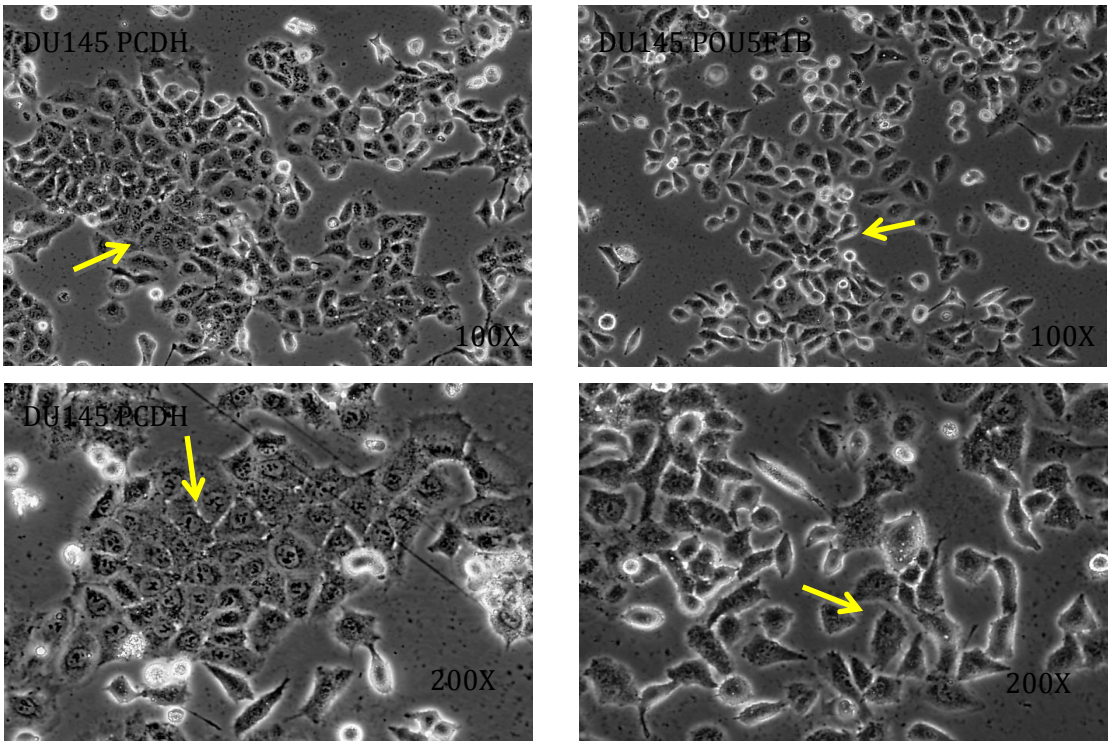


Figure 5. Comparasion of cell morphology between DU145 pCDH myc vector and DU145 POU5F1B cells. The tight cell-cell adherent junctions were found in DU145 pCDH myc vector cells, but were reduced in DU145 POU5F1B cells.

1.6. POU5F1B can induce EMT in DU145 cells

We observed the cells lost tight cell-cell adhesion when POU5F1B is overexpressed, which suggests the cells undergo EMT process. Down-regulation of E-Cadherin is one of the hallmarks of EMT. First, we assessed the cellular localization and expression of E-Cadherin in DU145 pCDH myc vector and DU145 POU5F1B cells. Immunocytochemistry staining showed typically E-Cadherin localization at cell-cell junction in pCDH myc vector cells, but such staining pattern was disapperaed in POU5F1B overexpressed cells (Figure 6 A). Furthermore, immunoblot confirmed the down-regulation of E-Cadherin after POU5F1B overexpression (Figure 6B). The reductidon of E-Cadherin was not found in immortalized normal prostate epithelial cell line RWPE-1 cells with POU5F1B overxpression (Figure 6B). This suggest that POU5F1B decrease E-Cadherin is cell-dependent. E-Cadherin is the main component of the cell-cell adhesion junctions, loss of its expression will increase cell mitility.

To investigate other EMT-related gene expression, we did EMT-related gene microarray. As showed in Figure 7, the eptithelila marker, CDH1 gene , which encoding E-Cadherin decreased almost 10 fold. The mesenchymal marker, CDH2, N-Cadherin encoding gene and CLDN1, claudin-1 encoding gene are increased. Twist, SIP-1 and Vimentin are the mesenchymal marker that are decreased in DU145 POU5F1B cells compared with DU145 pCDH cells.

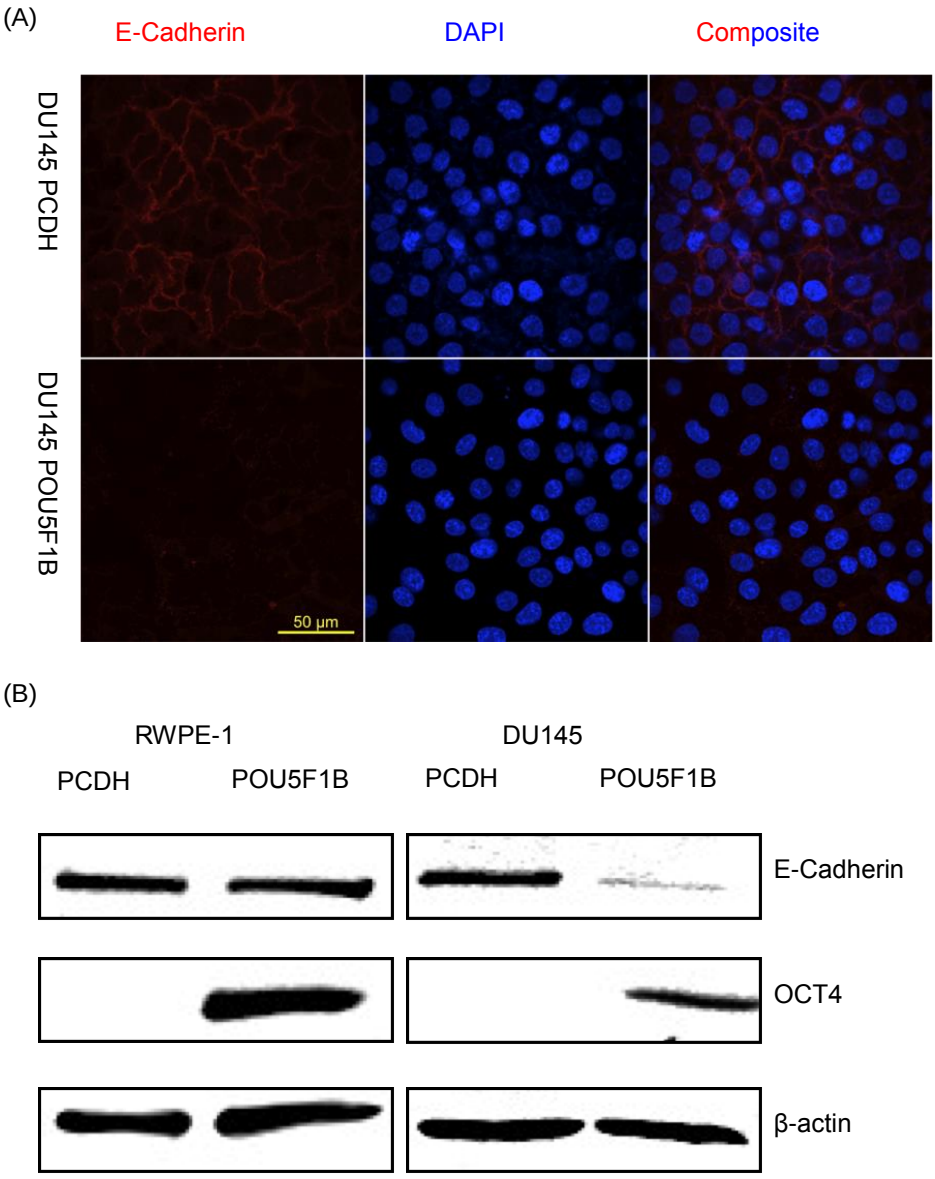


Figure 6. Effect of POU5F1B on E-Cadherin cellular localization and expression.

(A) Immunocytochemistry of E-Cadherin in DU145 pCDH and DU145 POU5F1B cells. POU5F1B causes loss of E-Cadherin localization at cell-cell junctions. DU145 pCDH myc vector and DU145 POU5F1B cells growed on coverslips were fixed, subjected to double staining with anti-E-Cadherin (red) and DAPI (blue) and examined by fluorescence microscopy.

(B) Immunoblot of E-Cadherin in RWPE-1 pCDH and POU5F1B, DU145 pCDH and POU5F1B cells. It was noticed that POU5F1B could decrease E-Cadherin expression in DU145 cells, but not in RWPE-1 cells.

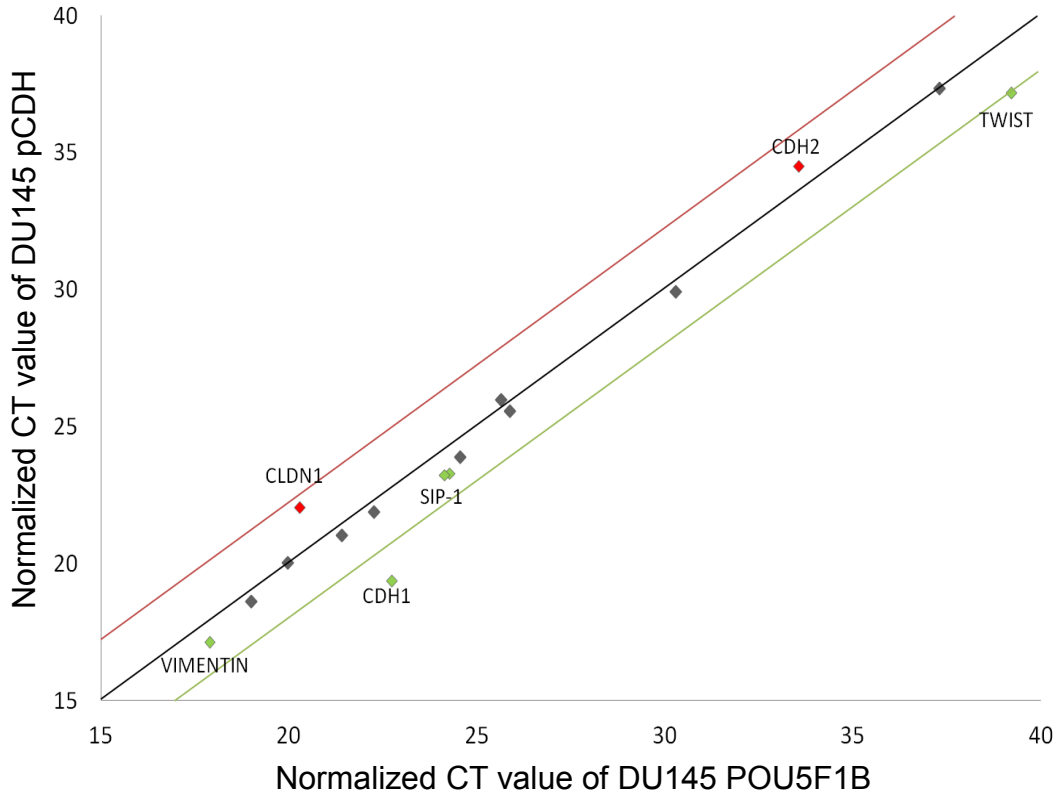


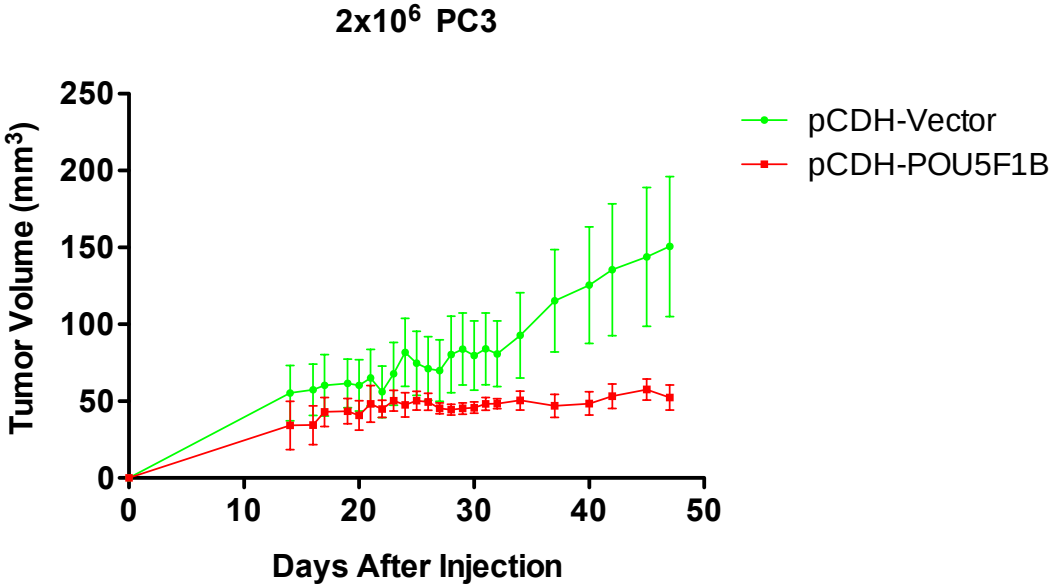
Figure 7. POU5F1B overexpression in DU145 regulates EMT-related genes expression. Scatter plot comparing EMT-related gene expression profiles between DU145 pCDH and DU145 POU5F1B cells. Red lines indicate fourfold increase in DU145 POU5F1B compared with DU145 pCDH cells. Green lines indicate fourfold decrease in DU145 POU5F1B compared with DU145 pCDH cells. CT value was normalized to beta-actin CT.

1.7. The effect of POU5F1B on tumor growth *in vivo*

In out last year report, we found POU5F1B, when overexpressed in LNCaP-T (FGC) cells, stimulated tumor growth. Due to prostate cancer heterogeneity, we wished to extend the findings to other prostate cancer cell lines. Surprisingly we found POU5F1B actullay supppressed the growth of tumors from PC3 or DU145 cells.

To determine whether POU5F1B promote tumor growth, we subcutaneously injected $2X10^6$ PC3 cells with pCDH vector or POU5F1B expression, DU145 cells with pCDH vector or POU5F1B expression into nude mice. 5 mice for each group. 4 out of 5 mice formed tumor in PC3 pCDH and PC3 POU5F1B cells after injection, but tumors formed in PC3 POU5F1B cells grows much slower than PC3 pCDH cells(Figure 13 A). In DU145 pCDH cells, 3 out of 5 mice formed tumors, and only 1 out of 5 mice formed tumor in DU145 POU5F1B cells. It is also showed that POU5F1B suppress tumor growth in DU145 cells (Figure 13 B). POU5F1B overexpressing cells exhibit stem cell like properties. The slower tumor growth that we observed in mice is likely the result of the higher proportion of quiescient pluripotent cells, and therefore lower proportions of highly proliferative cells, that make up the POU5F1B population.

A)



(B)

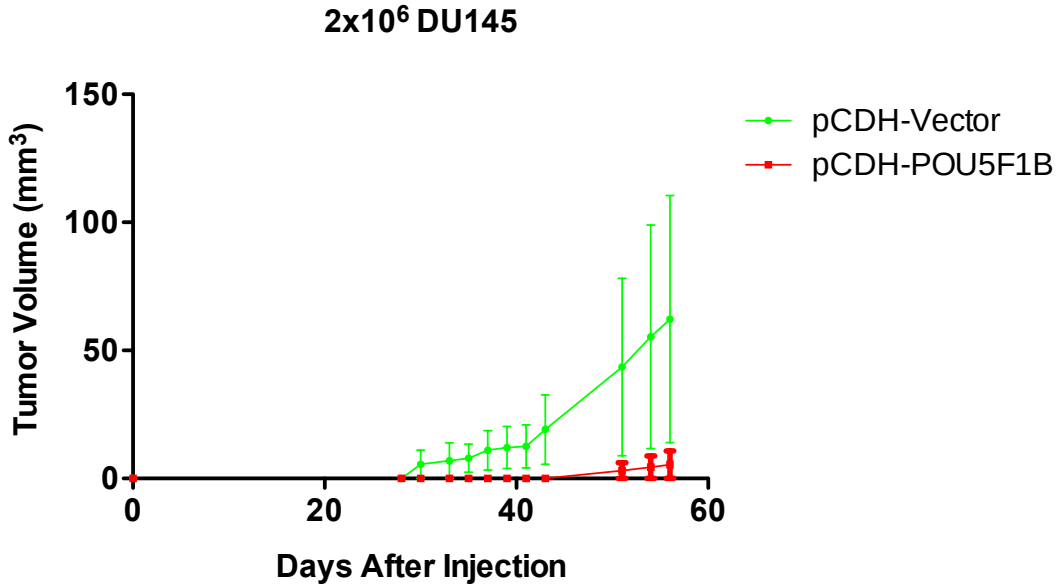


Figure 8. Suppression of tumor growth by POU5F1B in PC3 and DU145 cells.

Task 2: Determine whether tumor Oct4 can be targeted to reduce prostate tumor formation, progression, and metastasis (Month 12 -30).

We have introduced shRNA constructs into PC3MM and DU145 cells. Currently we are screening stable clones that can be used for our studies.

Task 3: Elucidate the mechanism involved for tumor Oct4 in promoting prostate carcinogenesis (Month 18 – 36).

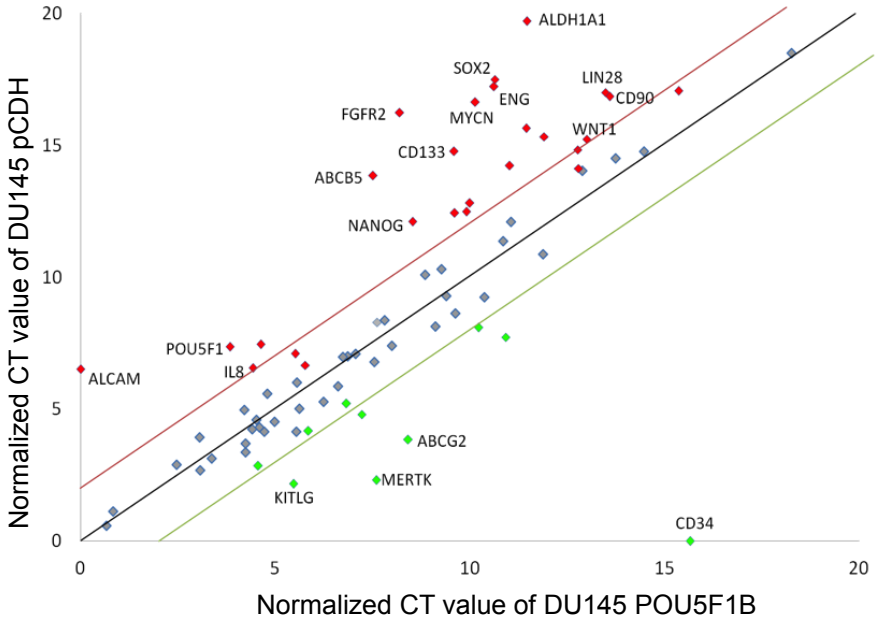
3.1. POU5F1B and stem cell

To investigate whether POU5F1B overrxpression can induce cancer stem cell-related genes expression, we did cancer stem cell microarray. 84 cancer stem cell related genes are investigated. The functional grouping of these genes were shown in Table 2. We observed a very prominent overexpression of cancer stem cell related genes after POU5F1B expression. In DU145 cells, nearly half of all tested genes were overexpressed by at least 2 folds in POU5F1B cells. In PC3 cells, 2 fold overexpression was observed in a quarter of tested genes. Most notably, we found 14 genes that are significantly overexpressed in both DU145 and PC3 POU5F1B cells, including essential pluripotency regulators NANOG, SOX2, and BMI; cell adhesion and migration molecules PECAM1, THY1, ITGA4; and oncogenes MYCN, cKIT, WNT1 (Figure 9).

Table 2. Functional grouping of cancer stem cell microarray genes

Functional group	Genes
Cancer Stem Cell Markers	ABCB5, ALCAM, ALDH1A1, ATXN1, BMI1, CD24, CD34, CD38, CD44, ENG, ETFA, FLOT2, GATA3, ITGA2, ITGA4, ITGA6, ITGB1, KIT, MS4A1, MUC1, PECAM1, PROM1, PTPRC, THY1
Proliferation	EGF, ERBB2, KITLG, LIN28B, NOS2
Self-Renewal	BMP7, DNMT1, FGFR2
Pluripotency	KLF4, LIN28A, MYC, NANOG, POU5F1, SOX2
Asymmetric Division	FOXP1, HDAC1, MYCN, SIRT1, WNT1.
Migration & Metastasis	AXL, ID1, IL8, KLF17, PLAT, PLAUR, SNAi1, TWIST1, TWIST2, ZEB1, ZEB2.
Loss of Stemness	ALDH1A1, CD34, DACH1, FOXA2, PECAM1, PTCH1.
Signal Transduction Pathways	
Hippo Signaling	LATS1, MERTK, SAV1, TAZ, WWC1, YAP1
Hedgehog Signaling	PTCH1, SMO.
Notch Signaling	DLL1, DLL4, JAG1, MAML1, NOTCH1, NOTCH2
WNT Signaling	DKK1, EPCAM, FZD7, WNT1
PI3K/AKT/mTOR signaling	ABCG2, GSK3B
STAT/NFκB Signaling	IKBKB, JAK2, NFKB1
Therapeutic Targets	ABCG2, ATM, AXL, CHEK1, DDR1, DKK1, EPCAM, FZD7, GSK3B, ID1, IKBKB, JAK2, KLF17, NFKB1, PTCH1, SMO, STAT3, TGFBR1, WEE1

(A)


ALDH1A1
SOX2
LIN28
ENG
CD90
FGFR2
MYCN
WNT1
CD133
ABCB5
NANOG
POU5F1
IL8
ALCAM
ABCG2
MERTK
KITLG
CD34
Normalized CT value of DU145 pCDH
Normalized CT value of DU145 POU5F1B
0
5
10
15
20

(B)

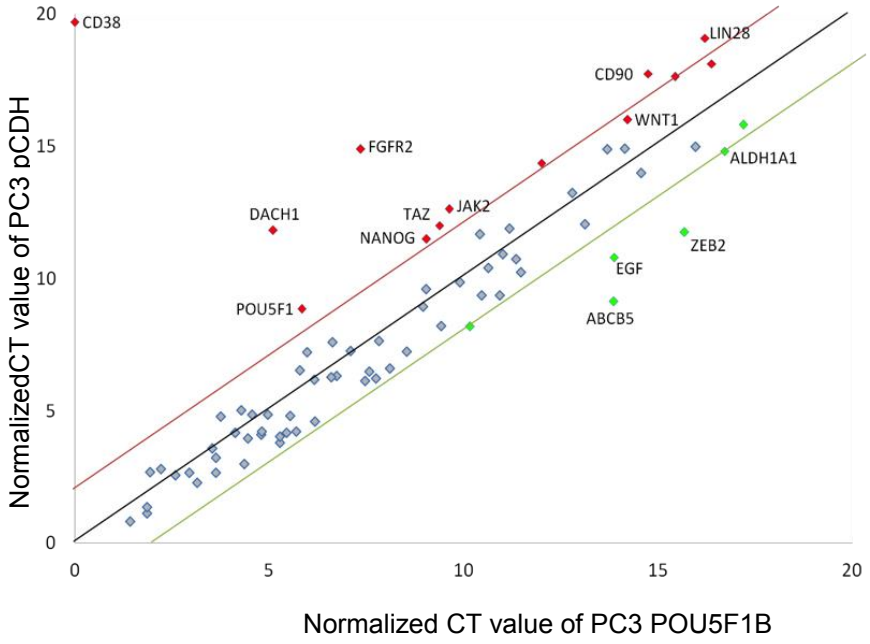

CD38
LIN28
CD90
WNT1
FGFR2
ALDH1A1
DACH1
TAZ
JAK2
NANOG
ZEB2
EGF
POU5F1
ABCB5
NormalizedCT value of PC3 pCDH
Normalized CT value of PC3 POU5F1B
0
5
10
15
20

Figure 9. Scatter plot of cancer stem cell genes expression profile between cells with pCDH vector and POU5F1B expression. Red lines indicate fourfold increase in POU5F1B compared with pCDH cells. Green lines indicate fourfold decrease in POU5F1B compared with pCDH cells. CT value was normalized to house-keeping gene CT.

(A) Cancer stem cell genes expression profile in DU145 pCDH and DU145 POU5F1B.

(B) Cancer stem cell genes expression profile in PC3 pCDH and PC3 POU5F1B.

KEY RESEARCH ACCOMPLISHMENT and REPORTABLE OUTCOMES

Presentations and abstracts:

An abstract has been submitted to Keystone scientific meeting regarding the suppression of PC3 and DU145 tumor growth by POU5F1B.

Research articles published:

The manuscript is still in the process of revisions.

Conclusions and significance (So what?):

The studies will determine whether tumor Oct4 causes epithelial-mesenchymal transition and stimulates tumor cell migration during tumor progression to metastatic disease. Surprisingly we found POU5F1B actually inhibited the growth of primary tumors from PC3 and DU145 cells (but not from LNCaP cells). The data suggest a complex role for tumor Oct4 in prostate cancer progression and metastasis. Further research is needed to determine whether tumor Oct4 as a target of intervention to eliminate tumorigenic and metastatic cells.

APPENDICES

N/A

SUPPORTING DATA

Embedded in the reporting body

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